FORM PTO-1390 (REV. 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	
0760-0290P	
U.S. APPLICATION NO. (If known, see 37 CFR 1.5)	09/856725 NEW
INTERNATIONAL APPLICATION NO.	PCT/JP00/06560
INTERNATIONAL FILING DATE	September 25, 2000
PRIORITY DATE CLAIMED	September 27, 1999

TITLE OF INVENTION
NUCLEIC ACID FRAGMENTS, RECOMBINANT VECTORS CONTAINING THE SAME AND METHOD FOR PROMOTING EXPRESSION OF STRUCTURAL GENES USING THE SAME

APPLICANT(S) FOR DO/EO/US
UEKI, Jun; MORIOKA, Shinji

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39 (1).
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau. WO 01/23544
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☒ is transmitted herewith.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4)
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 20. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98./International Search Report with cited references
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☒ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821-1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☐ Other items or information:

09856725 .062301

JC18 Rec'd PCT/PTO 25 MAY 2001

U.S. APPLICATION NO (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/856725 NEW	PCT/JP00/06560	0760-0290P

21. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO. **$1,000.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$860.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO. **$710.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$690.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4). **$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	24 - 20 =	2	X **$18.00**	$ 72.00	
Independent Claims	2 - 3 =	0	X **$80.00**	$ 0	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)	YES		+ **$270.00**	$ 270.00	
TOTAL OF ABOVE CALCULATIONS =				$ 1202.00	
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				$ 0	
SUBTOTAL =				$ 1202.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐20 ☐30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$ 0	
TOTAL NATIONAL FEE =				$ 1202.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$ 0	
TOTAL FEES ENCLOSED =				$ 1202.00	
				Amount to be: refunded	$
				charged	$

a. ☒ A check in the amount of $ **1202.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account. No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-2448.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

Send all correspondence to:
Birch, Stewart, Kolasch & Birch, LLP or Customer No. 2292
P.O. Box 747
Falls Church, VA 22040-0747
(703)205-8000

Date: May 25, 2001

By _____ #34868
for Gerald M. Murphy, Jr., #28,977

/ja

09/856725

JC18 Rec'd PCT/PTO 25 MAY 2001

PATENT
0760-0290P

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant:	UEKI, Jun et al.	Conf.:
Int'l. Appl. No.:	PCT/JP00/06560	
Appl. No.:	New	Group:
Filed:	May 25, 2001	Examiner:
For:	NUCLEIC ACID FRAGMENTS, RECOMBINANT VECTORS CONTAINING THE SAME AND METHOD FOR PROMOTING EXPRESSION OF STRUCTURAL GENES USING THE SAME	

PRELIMINARY AMENDMENT

BOX PATENT APPLICATION
Assistant Commissioner for Patents
Washington, DC 20231

May 25, 2001

Sir:

The following Preliminary Amendments and Remarks are respectfully submitted in connection with the above-identified application.

AMENDMENTS

IN THE SPECIFICATION:

Please amend the specification as follows:

Before line 1, insert --This application is the national phase under 35 U.S.C. § 371 of PCT International Application No. PCT/JP00/06560 which has an International filing date of September 25, 2000, which designated the United States of America and was published in Japanese. --

Docket No. 0760-0290P

REMARKS

The specification has been amended to provide a cross-reference to the previously filed International Application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By _____
Gerald M. Murphy, Jr., #28,977

P.O. Box 747
Falls Church, VA 22040-0747
(703) 205-8000

GMM/ja
0760-0290P

(Rev. 02/12/01)

PATENT
0760-0290P

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant:	UEKI, Jun et al.	Conf.:	3445
Appl. No.:	09/856,725	Group:	UNASSIGNED
Filed:	May 25, 2001	Examiner:	UNASSIGNED

For: NUCLEIC ACID FRAGMENTS, RECOMBINANT VECTORS CONTAINING THE SAME AND METHOD FOR PROMOTING EXPRESSION OF STRUCTURAL GENES USING THE SAME.

AMENDMENT

Honorable Commissioner of Patents
Washington, D.C. 20231

September 4, 2001
(Tuesday after Holiday)

Sir:

In response to the Notice to Comply with Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures mailed July 2, 2001, the following Amendments and Remarks are respectfully submitted.

IN THE SPECIFICATION

Please replace the text on page 6, lines 6 and 7 with the following amended text:

5'-aagtcccccg ggccgcgcca gcggaag-3' (SEQ ID NO: 4)

3'-gacacccaca gccgtctata gttcgta-5' (SEQ ID NO: 6)

Please replace the Sequence Listing of record with the Substitute Sequence Listing enclosed herewith.

Application No. 09/856,725

REMARKS

Enclosed herewith is a Substitute Sequence Listing to be inserted into the specification as indicated above. The Substitute Sequence Listing in no way introduces new matter into the specification. The Substitute Sequence Listing now includes SEQ ID NO: 6 which appears as a PCR primer on page 6 of the specification. Also submitted herewith is a computer readable form of the Substitute Sequence Listing. The computer readable form of the Substitute Sequence Listing, file "0760-0290P.ST25.txt", is identical to the paper copy, except that it lacks formatting.

Attached hereto is a marked-up version of the changes made to the specification by this amendment.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. §1.16 or under 37 C.F.R. §1.17; particularly, extension of time fees.

Respectfully submitted,
BIRCH, STEWART, KOLASCH & BIRCH, LLP

By ______ #36,623
Gerald M. Murphy, Jr., #28,977
P.O. Box 747
Falls Church, VA 22040-0747
(703) 205-8000

GMM/BCF
Enclosures: Paper and Disk Copy of the Substitute Sequence Listing
Copy of the Notice to Comply, Version with Markings

Version With Markings to Show Changes Made

The text on page 6, lines 6 and 7 is amended as shown:

5'-aagtcccccg ggccgcgcca gcggaag-3' (SEQ ID NO: 4)

3'-gacacccaca gccgtctata gttcgta-5' (SEQ ID NO: 6)

SEQUENCE LISTING

<110> Jun UEKI et al.

<120> Nucleic acid fragments, recombinant vectors containing the same and method for promoting expression of structural genes using the same.

<130> 0760-0290P

<140> US 09/856,725
<141> 2001-05-25

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SPECIFICATION

Nucleic Acid Fragments, Recombinant Vectors Containing the Same and Method for Promoting Expression of Structural Genes Using the Same

Technical Field

The present invention relates to a nucleic acid fragment having an activity to promote expression of a structural gene located at a downstream site thereof, a recombinant vector containing the same, and to a method for expressing the structural gene using the same, as well as to a plant in which expression of a desired structural gene is promoted by the method.

Background Art

Promotion of foreign gene expression is the most required technique in applying the genetic engineering technique to plants. One of the techniques is the utilization of DNA fragments. Known DNA fragments which promote expression of foreign genes include some introns (Simpson and Filipowicz 1996. Plant Mol.Biol. 32: 1-41) including an intron of maize alcohol dehydrogenase (Callis et al. Gene & Development 1, 1183-1200 (1987)), as well as the first intron of rice phospholipase D (WO96/30510). Influences of deletion of a part of inner regions of DNA fragments derived from introns, and of insertion of the same intron into the intron, on the promotion of expression have been reported (Mascarenhas et al. Plant Mol. Biol. 15, 913-920 (1990), Clancy et al. Plant Sci.98, 151-161 (1994)).

However, so far, types of available DNA fragments are limited. Further, actions of the DNA fragments vary depending on the type of the plant, and vary depending on the organs or tissues even in the same plant (Simpson and Filipowicz 1996. Plant Mol.Biol. 32: 1-41). Therefore, existence of DNA fragments exhibiting various types of expression-promotion actions is desired.

Disclosure of the Invention

Accordingly, an object of the present invention is to provide a novel nucleic

acid fragment having an activity to promote expression of a structural gene located downstream thereof, and to provide a method for promoting expression of the structural gene downstream thereof.

The present inventors intensively studied to discover that the second intron of rice PLD gene has a high activity to promote gene expression, thereby completing the present invention.

That is, the present invention provides a nucleic acid fragment having the nucleotide sequence shown in SEQ ID NO:1 in the Sequence Listing, or having the same nucleotide sequence as shown in SEQ ID NO:1 except that one or more nucleotides are substituted or deleted, or one or more nucleotides are inserted therein or added thereto, which has an activity to promote expression of a structural gene located downstream thereof. The present invention also provides a nucleic acid fragment having the nucleotide sequence shown in SEQ ID NO:1 in the Sequence Listing, or a nucleic acid fragment which hybridizes with the nucleic acid fragment under stringent conditions, which has an activity to promote expression of a structural gene located downstream thereof. The present invention further provides a recombinant vector which contains the above-described nucleic acid fragment according to the present invention and a structural gene located downstream of the nucleic acid fragment, by which expression of the structural gene is promoted by the nucleic acid fragment. The present invention further provides a method for promoting expression of a structural gene comprising inserting the nucleic acid fragment according to the present invention into a site upstream of the structural gene. The present invention further provides a plant in which expression of a desired structural gene is promoted, and progenies thereof retaining the character.

By the present invention, a novel nucleic acid fragment having a high activity to promote expression of a structural gene was provided. As is apparent from the Example below, the activity of the nucleic acid fragment according to the present

invention to promote expression of the structural gene downstream thereof is much larger than that of the known first intron of rice PLD gene, which has the similar function. Therefore, by inserting the nucleic acid fragment of the present invention into a site upstream of the structural gene, expression of the structural gene is much more promoted. Thus, by the present invention, for example, expression of a foreign gene using a recombinant vector may be much more promoted, so that the present invention will make a large contribution in the field of genetic engineering.

Best Mode for Carrying out the Invention

As mentioned above, the nucleic acid fragment according to the present invention is a nucleic acid fragment having the nucleotide sequence shown in SEQ ID NO:1 in the Sequence Listing, or having the same sequence as shown in SEQ ID NO:1 except that one or more nucleotides are substituted or deleted, or one or more nucleotides are inserted therein or added thereto, which has an activity to promote expression of a structural gene located downstream thereof.

As mentioned above, the nucleic acid fragments (hereinafter also referred to as "modified nucleic acid fragment" for convenience) having the same nucleotide sequence as shown in SEQ ID NO: 1 except that one or a plurality of nucleotides are substituted or deleted, or except that one or a plurality of nucleotides are inserted or added, which have activities to promote expression of a structural gene located downstream of the nucleic acid fragments are also within the scope of the present invention. In this case, the region in the modified nucleic acid fragment, which corresponds to a region in the sequence shown in SEQ ID NO:1 preferably has a homology of not less than 70%, more preferably not less than 85%, more preferably not less than 95% with the sequence shown in SEQ ID NO:1. The homology of the nucleotide sequence may easily be calculated by using a well-known software such as FASTA. Further, these modified nucleic acid fragments preferably hybridize with the nucleic acid having the nucleotide sequence shown in SEQ ID NO: 1 under

stringent conditions (i.e., hybridization is carried out in an ordinary hybridization solution such as 5 x Denhardt's reagent, 6 x SSC, 0.5% SDS or 0.1% SDS, at 50 to 65°C, preferably in two steps at 50°C and at 60°C, or in four steps at 50°C, 55°C, 60°C and 65°C).

The nucleic acid fragments each of which is a part of the nucleic acid fragment having the nucleotide sequence shown in SEQ ID NO:1, which have activities to promote expression of a structural gene located downstream of the nucleic acid fragments are also within the scope of the present invention. Further, nucleic acid fragments obtained by ligating a plurality of the nucleic acid fragments according to the present invention are also within the scope of the present invention. In this case, the nucleic acid fragments according to the present invention may be directly ligated or an intervening sequence may exist therebetween.

The nucleic acid according to the present invention may be either DNA or RNA. However, DNA is preferred in view of stability.

Since the nucleotide sequence of the nucleic acid fragment according to the present invention has been determined by the present invention and since the nucleic acid fragment is originated from the genome of rice, the nucleic acid fragment may easily be prepared by a nucleic acid-amplification method such as PCR using the genomic DNA of rice as the template. PCR is well-known in the art and a kit and apparatus therefor are commercially available, so that it can be easily carried out. Further, the above-mentioned modified nucleic acid fragments may be obtained by subjecting the thus obtained nucleic acid fragment to the well-known site-specific mutagenesis.

In cases where a plurality of nucleic acid fragments according to the present invention are ligated, a plurality of nucleic acid fragments according to the present invention may be preliminarily ligated, or a nucleic acid fragment according to the present invention may be inserted into a region containing the nucleic acid fragment

according to the present invention.

By inserting the above-described nucleic acid fragment according to the present invention to a site upstream of a structural gene, the expression of the structural gene may be promoted. Structural genes are controlled by a promoter located upstream thereof. The nucleic acid fragment according to the present invention may be inserted either between the promoter and the structural gene or at a site upstream of the promoter, and the former is preferred. In this case, the distance between the nucleic acid fragment according to the present invention and the structural gene may preferably be 0 bp to 1000 bp, and the distance between the promoter and the nucleic acid fragment according to the present invention may also preferably be 0 bp to 1000 bp.

The present invention also provides recombinant vectors obtained by applying the above-described method of the present invention to an expression vector. The recombinant vector according to the present invention may easily be prepared by inserting the nucleic acid fragment according to the present invention and a structural gene of which expression is to be promoted into a cloning site of a commercially available expression vector. Such an expression vector may preferably be one for plants. Various expression vectors for plants are well-known in the art and commercially available. These expression vectors include a replication origin for replication in host cells, a promoter, cloning sites giving restriction sites for inserting foreign genes, and a selection marker such as a drug resistant gene, and usually contain a terminator which stably terminates transcription. In the method of the present invention, any of these known expression vectors may be employed.

Example

The present invention will now be described more concretely by way of examples thereof. It should be noted that the present invention is not restricted to the Example.

A DNA fragment having the second intron of rice PLD gene and 37mer exon regions at both ends of the second intron (the nucleotide of this DNA fragment is shown in SEQ ID NO:2 in Sequence Listing) was amplified by PCR using the following primers and a known rice genomic clone (SEQ ID NO:5 of WO95/0934) as the template.

5'-aagtcccccgggccgcgccagcggaag-3'

3'-gacacccacagccgtctatagttcgta-5'

The obtained fragments amplified by PCR were digested with Sma I and Eco RV and inserted into the Sma I site of a vector pBI221 commercially available from CLONTECH, which contains a β-glucuronidase (GUS) gene at a downstream site of 35S promoter. Transient expression of the gene was examined by the method of Sheen (Sheen 1991, Plant Cell 3:225-245). That is, the constructed plasmids were introduced into protoplasts isolated from etiolated maize leaves by electroporation, and transient expression of GUS was measured by the above-mentioned method.

For comparison, the first intron (SEQ ID NO:3 in Sequence Listing) of rice PLD gene was amplified by PCR by the method described in WO96/30510 and inserted into the Sma I site of pBI221, followed by introduction of the obtained vector into maize in the similar manner as described above. The expression of GUS was determined. The results are shown in Table 1 below.

Table 1

Plasmid	GUS Activity (4-MU pmol/10^7 cells/min)
pBI221 (35S promoter, GUS)(Comparative Example)	140
pBI221 + PLD first intron (Comparative Example)	630
pBI221 + PLD second intron (Example)	11,000

As is apparent from these results, the activity of the nucleic acid fragment according to the present invention to promote expression of the structural gene located downstream thereof is much higher than that of the first intron of rice PLD, which is known to have the similar function.

CLAIMS

1. A nucleic acid fragment having the nucleotide sequence shown in SEQ ID NO:1 in the Sequence Listing, or having the same nucleotide sequence as shown in SEQ ID NO:1 except that one or more nucleotides are substituted or deleted, or one or more nucleotides are inserted therein or added thereto, which has an activity to promote expression of a structural gene located downstream thereof.

2. The nucleic acid fragment according to claim 1, which has a nucleotide sequence homology of not less than 70% to the nucleotide sequence shown in SEQ ID NO:1 in the Sequence Listing.

3. A nucleic acid fragment having the nucleotide sequence shown in SEQ ID NO:1 in the Sequence Listing, or a nucleic acid fragment which hybridizes with the nucleic acid fragment under stringent conditions, which has an activity to promote expression of a structural gene located downstream thereof.

4. The nucleic acid fragment according to claim 1, which has the nucleotide sequence shown in SEQ ID NO:1 in the Sequence Listing or a part thereof that has an activity to promote expression of a structural gene located downstream thereof.

5. The nucleic acid fragment according to claim 4, which has the nucleotide sequence shown in SEQ ID NO:1 in the Sequence Listing.

6. The nucleic acid fragment according to claim 1, which has the nucleotide sequence shown in SEQ ID NO:2 in the Sequence Listing.

7. A recombinant vector comprising said nucleic acid fragment according to any one of claims 1 to 6, and a structural gene located downstream of said nucleic acid fragment, by which expression of said structural gene is promoted by said nucleic acid fragment.

8. A method for promoting expression of a structural gene comprising inserting said nucleic acid fragment according to any one of claims 1 to 6 into a site upstream of said structural gene.

9. A plant in which expression of a desired structural gene is promoted by the method according to claim 7, or a progeny thereof retaining the character.

ABSTRACT

A novel nucleic acid fragment which has an high activity to promote expression of a structural gene located downstream thereof, and a method for promoting expression of the structural gene located downstream thereof using the nucleic acid are disclosed. A nucleic acid fragment having the nucleotide sequence shown in SEQ ID NO:1 in the Sequence Listing, and nucleic acid fragments having the same sequence as shown in SEQ ID NO:1 except that one or more nucleotides are substituted or deleted, or one or more nucleotides are inserted therein or added thereto, which have activities to promote expression of a structural gene located downstream thereof were provided.

Attorney Docket No.

BIRCH, STEWART, KOLASCH & BIRCH, LLP
P.O. Box 747 · Falls Church, Virginia 22040-0747
Telephone: (703) 205-8000 · Facsimile: (703) 205-8050

COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT AND DESIGN APPLICATIONS

As a below named inventor, I hereby declare that: my residence, post office address and citizenship are as stated next to my name; that I verily believe that I am the original, first and sole inventor (if only one inventor is namedbelow) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:"Nucleic Acid Fragments, Recombinant Vectors Containing the Same and Method for Promoting Expression of Structural Genes Using the Same"

the specification of which is attached hereto. If not attached hereto,
the specification was filed on _____ as
United States Application Number _____;
and amended on _____ (if applicable) and/or
the specification was filed on 09/25/00 as PCT
International Application Number PCT/JP00/06560; and was
amended under PCT Article 19 on _____ (if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I do not know and do not believe the same was ever known or used in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to this application, that the same was not in public use or on sale in the United States of America more than one year prior to this application, that the invention has not been patented or made the subject of an inventor's certificate issued before the date of this application any country foreign to the United States of America on an application filed by me or my legal representative or assigns more than twelve months (six months for designs) prior to this application, and that no application for patent or inventor's certificate on this invention has been filed in any country foreign to the United States of America prior to this application by me or my legal representatives or assigns, except as follows.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed
271762/99 (Number)	Japan (Country)	09/27/99 (Month/Day/Year Filed)	Yes
_____ (Number)	_____ (Country)	_____ (Month/Day/Year Filed)	

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional applications(s) listed below.

_____ (Application Number)	_____ (Filing Date)
_____ (Application Number)	_____ (Filing Date)

All Foreign Applications, if any, for any Patent or Inventor's Certificate Filed More than 12 Months (6 Months for Designs) Prior to the Filing Date of This Application:

Country	Application Number	Date of Filing (Month/Day/Year)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States and/or PCT application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States and/or PCT application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to the patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

_____ (Application Number)	_____ (Filing Date)	_____ (Status - patented, pending, abandoned)
_____ (Application Number)	_____ (Filing Date)	_____ (Status - patented, pending, abandoned)

09856725 .062101

Attorney Docket No.

I hereby appoint the following attorneys to prosecute this application and/or an international application based on this application and to transact all business in the Patent and Trademark Office connected therewith and in connection with the resulting patent based on instructions received from the entity who first sent the application papers to the attorneys identified below, unless the inventor(s) or assignee provides said attorneys witha written notice to the contrary:

(18)

Raymond C. Stewart	(Reg. No. 21,066)	Terrell C. Birch	(Reg. No. 19,382)
Joseph A. Kolasch	(Reg. No. 22,463)	James M. Slattery	(Reg. No. 28,380)
Bernard L. Sweeney	(Reg. No. 24,448)	Michael K. Mutter	(Reg. No. 29,680)
Charles Gorenstein	(Reg. No. 29,271)	Gerald M. Murphy, Jr.	(Reg. No. 28,977)
Leonard R. Svensson	(Reg. No. 30,330)	Terry L. Clark	(Reg. No. 32,644)
Andrew D. Meikle	(Reg. No. 32,868)	Marc S. Weiner	(Reg. No. 32,181)
Joe McKinney Muncy	(Reg. No. 32,334)	Donald J. Daley	(Reg. No. 34,313)
John W. Bailey	(Reg. No. 32,881)	John A. Castellano	(Reg. No. 35,094)
Gary D. Yacura	(Reg. No. 35,416)		

Send Correspondence to:

BIRCH, STEWART, KOLASCH & BIRCH, LLP or **Customer No. 2292**
P.O. Box 747 · Falls Church, Virginia 22040-0747
Telephone: (703) 205-8000 · Facsimile: (703) 205-8050

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-00

GIVEN NAME/FAMILY NAME	INVENTOR'S SIGNATURE	DATE*
Jun UEKI	Jun Ueki	May 22, 2001
Residence (City, State & Country): **Iwata-gun, Shizuoka, JAPAN**		CITIZENSHIP: **Japanese** JPX
POST OFFICE ADDRESS (Complete Street Address including City, State & Country): **c/o Japan Tobacco Inc., Plant Breeding and Genetics Research Laboratory, 700, Higashibara, Toyoda-cho, Iwata-gun, Shizuoka 438-0802 JAPAN**		

2-00

GIVEN NAME/FAMILY NAME	INVENTOR'S SIGNATURE	DATE*
Shinji MORIOKA	Shinji Morioka	May 22, 2001
Residence (City, State & Country): **Iwata-gun, Shizuoka, JAPAN**		CITIZENSHIP: **Japanese** JPX
POST OFFICE ADDRESS (Complete Street Address including City, State & Country): **c/o Japan Tobacco Inc., Plant Breeding and Genetics Research Laboratory, 700, Higashibara, Toyoda-cho, Iwata-gun, Shizuoka 438-0802 JAPAN**		

GIVEN NAME/FAMILY NAME	INVENTOR'S SIGNATURE	DATE*
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GIVEN NAME/FAMILY NAME	INVENTOR'S SIGNATURE	DATE*
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POST OFFICE ADDRESS (Complete Street Address including City, State & Country)		

GIVEN NAME/FAMILY NAME	INVENTOR'S SIGNATURE	DATE*
Residence (City, State & Country)		CITIZENSHIP
POST OFFICE ADDRESS (Complete Street Address including City, State & Country)		

*DATE OF SIGNATURE

SEQUENCE LISTING

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